

Synthesis, Characterisation and DNA Binding Studies of Metal Complexes of Norfloxacin

S. PUSHPALATHA

Department of Chemistry, Noorul Islam University,
Kumaracoil, KK Dist, Tamilnadu, 629180, India
pushpa07latha@gmail.com

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Abstract: Metal complexes of tioconazole such as $[\text{Cu}(\text{nor})_2(\text{H}_2\text{O})_2]$, $[\text{Zn}(\text{nor})_2(\text{H}_2\text{O})_2]$, $[\text{Ni}(\text{nor})_2(\text{H}_2\text{O})_2] \cdot 4\text{H}_2\text{O}$ and $[\text{Fe}(\text{nor})_2(\text{H}_2\text{O})\text{Cl}] \cdot 5\text{H}_2\text{O}$ have been synthesized and characterised by elemental analysis, IR, UV-Visibe, ESR, NMR, XRD spectroscopic techniques. Thermal analysis was studied by TGA, DSC method. Morphological studies were carried out by scanning electron microscopic method. DNA binding studies were carried out by viscosity method. From the spectral studies an octahedral geometry was proposed for the copper complex. Prepared complexes showed enhanced DNA binding tendency than the parent drug that might be of interest for future research.

Keywords: Metal complexes, DNA binding studies, Viscosity method, Ligand

Introduction

Norfloxacin(1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1-*H*-quinolinecarboxylic acid) belongs to fluoroquinolones and is a wide-ranging drug used in treating bacterial infections of the urinary tract, respiratory tract and the skin. It is also known that NOR can be effective in treating diarrhoea and could treat conjunctivitis when it was administered in the form of eye drops. Norfloxacin is not effective against infections involving anaerobic bacteria (e.g. yeast, athlete's foot)¹⁻⁵. Quinolone antibiotics could participate in the formation of complexes in a number of ways⁶⁻⁹. In acidic media, quinolones are usually singly or doubly protonated making them unable to coordinate to the metal cations and, in such cases, only electrostatic interaction are observed between the drug and the metal ions¹⁰⁻¹². It is found that neutral quinolones in the zwitterionic state are capable of forming simple complexes (bidentate chelating)¹³⁻¹⁴. The quinolones could also act as bridging ligands and are capable of forming polynuclear complexes¹⁵.

Experimental

Synthesis of metal complexes

Complexes with the general formula $[\text{M}(\text{nor})_2]\text{X}_2 \cdot n\text{H}_2\text{O}$ synthesized, employing a 1:2 (metal ions:nor) ratio. A solution of 0.1 M of a salt of each Zn(II), Cu(II), Ni(II) and Fe(III)

previously dissolved in 10 mL of distilled water was added to a solution of 0.1 M of norfloxacin in 20 mL of acetone. The resulting mixtures were heated to 50 °C under reflux on a water bath for about 2 hours and then cooled. The complexes obtained were separated from the reaction mixture by filtration, washed with boiling water and acetone and dried under vacuum over CaCl₂. The complexes are formulated as [Cu(nor)₂(H₂O)₂], [Zn(nor)₂(H₂O)₂], [Ni(nor)₂(H₂O)₂].4H₂O and [Fe(nor)₂(H₂O)Cl].5H₂O.

Results and Discussion

IR spectrum

The position and/or the intensities of the peaks in the drugs are expected to be changed upon chelation. The $\nu(\text{OH})$, $\nu(\text{C}=\text{O})$, $\nu_{\text{asym}}(\text{COO})$ and $\nu_{\text{sym}}(\text{COO})$ stretching vibrations are observed at 3448, 1727, 1590 and 1396 cm⁻¹ for free ligand respectively. The participation of the carboxylate oxygen atom in the complex formation is evidenced from the shift in position of these bands to 3276-3427, 1709-1720 cm⁻¹ or the disappearance of the bands between 1549 cm⁻¹ – 1591 cm⁻¹ and 1381 cm⁻¹, 1394 cm⁻¹ for drug-metal complexes. The carbonyl $\nu(\text{C}=\text{O})$, stretching vibration is found in the free ligand at 1720 cm⁻¹. This band is shifted to lower wave numbers (1621 cm⁻¹, 1632 cm⁻¹) in the complexes indicating the participation of the carbonyl oxygen in coordination. New bands are found in the spectra of the complexes in the regions 524-555, 497-523 and 464-498 nm which are assigned to $\nu(\text{M}-\text{O})$ stretching vibrations of coordinated water, carboxylate-O and carbonyl-O, respectively¹⁶.

Electronic spectrum

The electronic spectra of the [Cu(nor)₂(H₂O)₂] complex showed two low-energy weak bands at 660-630 nm and a strong high-energy band at 330-328 nm. The low-energy band in this position is expected for an octahedral configuration and may be assigned to 10 Dq corresponding to the transition ²E_g→²T_{2g}. The strong high-energy band, in turn, is assigned to metal → ligand charge transfer. Also, the magnetic moment values (1.9-2.2 BM) for the copper(II) are indicative of antiferromagnetic spin-spin interaction through molecular association. Hence, the copper(II) complexes appear to be in the octahedral geometry with d_{x²-y²} ground state¹⁷. The Zn(II) complex showed no d-d bands as is expected for a d¹⁰ system and was found to be diamagnetic in nature. On the basis of analytical, conductance and spectroscopic data, Zn(II) complex is assigned an octahedral geometry. The electronic spectrum of Ni(II) complex showed three bands at 622, 382 and 308-265 nm corresponding to the transitions ³A_{2g}(F)→³T_{2g}(v₁), ³A_{2g}(F)→³T_{1g}(F) (v₂) and ³A_{2g}(F)→³T_{1g}(P)(v₃). The magnetic moment of Ni(II) complex is lying within the range of 2.5-3.5 B.M for octahedral Ni(II) complexes¹⁸. From the spectral data of the iron(III) complexes it can be seen that all of them exhibit one band at 508-568 nm which can be assigned to ⁶A_{1g} → ⁴T_{1g} transition characteristic of octahedral structure. The broad intense and poorly resolved bands between 320-450 nm may be assigned to MLCT¹⁹. The high intensity band below 320 nm is of ligand origin assignable to intraligand n - π / π - π* transition²⁰.

¹H NMR spectrum

The ¹H NMR spectrum further supports the assignment of the coordination modes. In the free ligand, the signal observed at 11 ppm can be assigned to the carboxylate -OH group. This signal disappears in the spectrum of the [Zn(nor)₂(H₂O)₂] complex, (Figure 1) which confirms the coordination of ligand to the M(II) ions through the deprotonated carboxylic -OH group. The peak at δ 3.55 ppm can be assigned as coming from the water molecules of

crystallization, which were not detected in the spectrum of the free ligand. The protons of the $-\text{CH}_2-$ group appears as a quartet at δ 4.80 ppm, while the $-\text{CH}_3$ group (triplet) at δ 3.5 ppm.

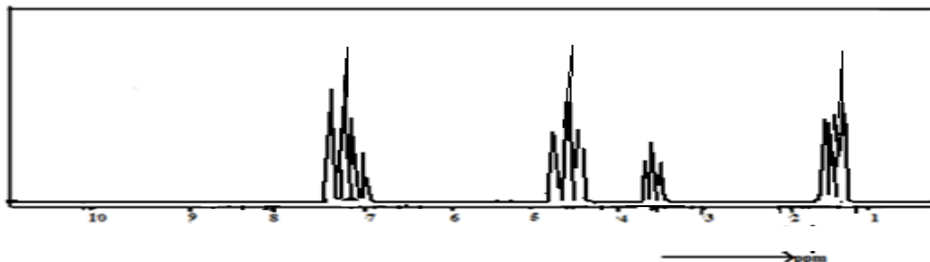


Figure 1. ^1H NMR spectrum $[\text{Zn}(\text{nor})_2(\text{H}_2\text{O})_2]$,

ESR spectrum

ESR spectrum of $[\text{Cu}(\text{nor})_2(\text{H}_2\text{O})_2]$ was studied under the magnetic field strength of 3000G. The spectrum of complex shows an intense absorption in the high field and is isotropic due to tumbling motion of the molecule. The 'g' values of the complex is g_{\parallel} (2.315) $>$ g_{\perp} (2.087) $>$ 2.0023, indicating that the unpaired electron in the ground state of Cu(II) ion is predominantly in $d_{x^2-y^2}$. The value of exchange interaction term G, estimated from the following expression is

$$G = g_{\parallel} - 2.0023 / g_{\perp} - 2.0023.$$

If $G > 4.0$, the local tetragonal axes are aligned parallel or only slightly misaligned. If $G < 4.0$, significant exchange coupling is present and misalignment is appreciable. The observed value for the exchange interaction term 'G' suggests that the complex has regular octahedral geometry²¹.

XRD studies

The XRD pattern of $[\text{Zn}(\text{nor})_2(\text{H}_2\text{O})_2]$ complex was studied in the 2θ range of 5-35°. The crystalline size of the complex was calculated from Scherer's formula. From the observed d_{XRD} pattern, the average crystalline size for the metallo drug is found to be 28 nm indicating that it is in nanocrystalline in nature.

TGA studies

The TGA studies were carried out to explore the thermal stability of the complexes. The thermal behaviour of the metal complex was studied in the temperature range of 25-800 °C. The TGA studies of complex $[\text{Fe}(\text{nor})_2(\text{H}_2\text{O})\text{Cl}].5\text{H}_2\text{O}$ reveal that the decomposition proceeds in three steps. In the first stage weight loss at 118 °C corresponds to the presence of the lattice water. The weight loss in the temperature range 130-220 °C is due to the presence of the coordinated water in the complex. A plateau was obtained after heating above 600 °C, which corresponds to the formation of stable metal oxide^{22,23}. The specific decomposition fragments are $\text{C}_{11}\text{H}_7\text{ON}_3\text{F}$ and CuO.

DSC studies

DSC behaviour of the metal complex (Figure 2) has been studied in the temperature range of 0-300 °C. The metal complex $[\text{Fe}(\text{nor})_2(\text{H}_2\text{O})\text{Cl}].5\text{H}_2\text{O}$ shows the single Tg at 261.4 °C. The copper complex shows a sharp endo thermal peak at 185.1 °C indicating the melting of the complex. The endothermic peak noted at 276.7 °C can be attributed to the decomposition of the complex. Broad exothermic peak obtained at 330 °C and 391.6 °C can also be due to the decomposition of the complex^{24,25}.

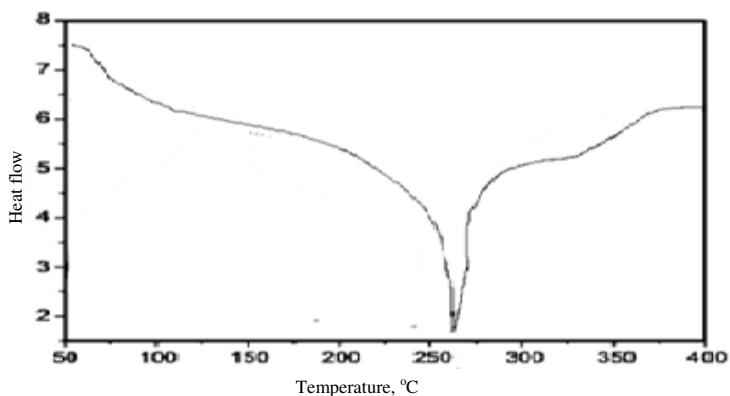


Figure 2. DSC graph of $[\text{Fe}(\text{nor})_2(\text{H}_2\text{O})\text{Cl}]\cdot 5\text{H}_2\text{O}$

SEM studies

Layers in the micrograph of $[\text{Fe}(\text{nor})_2(\text{H}_2\text{O})\text{Cl}]\cdot 5\text{H}_2\text{O}$ and $[\text{Ni}(\text{nor})_2(\text{H}_2\text{O})_2]\cdot 4\text{H}_2\text{O}$ reveal that the system (Figure 3 & 4) contains atoms in a well-defined pattern. Thus reactants have reacted completely to form a clear homogenous compound. In general the SEM photograph shows single phase formation with well-defined grain like shape and particle size in the range of 0.5 μm .

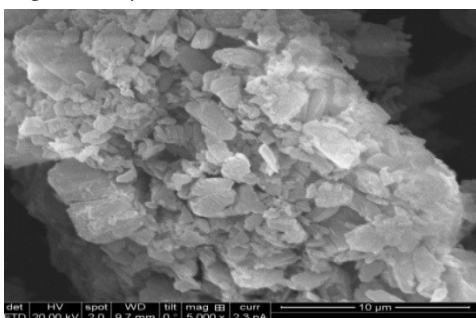


Figure 3. $[\text{Fe}(\text{nor})_2(\text{H}_2\text{O})\text{Cl}]\cdot 5\text{H}_2\text{O}$

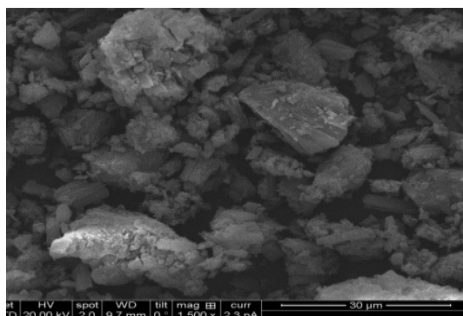


Figure 4. $[\text{Ni}(\text{nor})_2(\text{H}_2\text{O})_2]\cdot 4\text{H}_2\text{O}$

DNA binding studies by viscosity method

Optical photophysical probes generally provide necessary, but insufficient clues to further clarify the interactions between the study complex and DNA. Hydrodynamic measurements that are sensitive to length change (*i.e.* viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of binding in solution in the absence of crystallographic structural data. A classical intercalation model demands that the DNA helix lengthens as base pairs are separated in order to accommodate the binding ligand, leading to an increase in DNA viscosity. In contrast, a partial, non-classical intercalation of compound could bend (or kink) the DNA helix, reducing its effective length and concomitantly, its viscosity²⁶. Viscosity experiment results clearly show that both the compounds can intercalate between adjacent DNA base pairs, causing an extension in the helix, and thus increase the viscosity of DNA. The effects of increasing concentration of metal complexes of norfloxacin on the relative viscosity $(\eta/\eta_0)^{1/3}$ of DNA is shown in Figure 5.

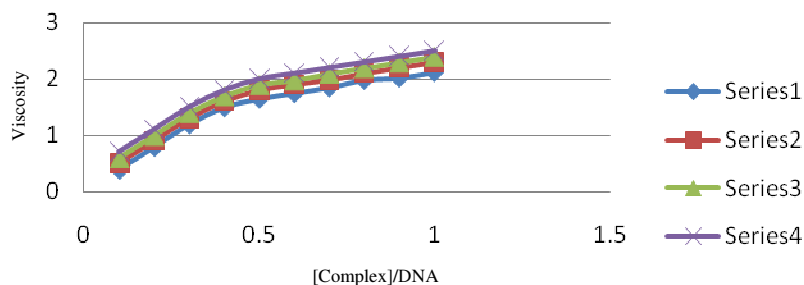


Figure 5. Effects of increasing concentration of metal complexes of norfloxacin on the relative viscosity $(\eta/\eta_0)^{1/3}$ of DNA

Conclusion

Mononuclear complexes of norfloxacin were characterized and characterized by various physico-chemical techniques. The overall geometry around respective metal ions has been drawn on the basis of electronic spectral and magnetic moment studies. It is hoped that the synthesis of these complexes may be recommended for a new line of search for new antibiotics. From the spectral studies the proposed geometry of the complex given in Figure 6.

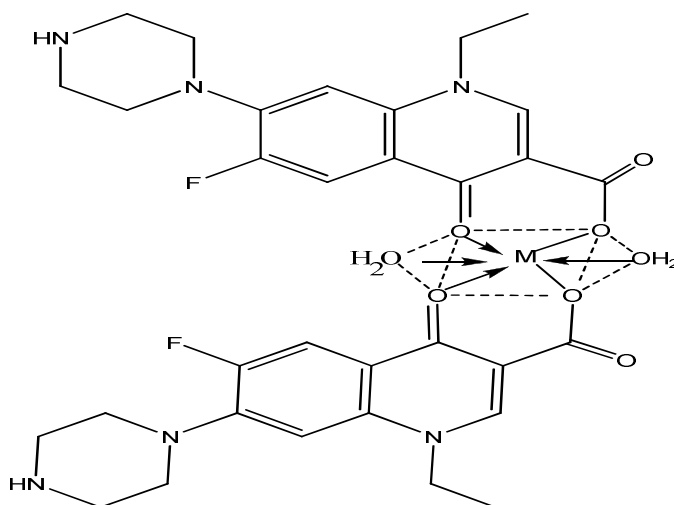


Figure 6. Geometry of copper complex of Norfloxacin, M=Cu(II) ion

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